Original Article

Simultaneous estimation of rosuvastatin calcium and hydrochlorthiazide from bulk and commercial products using a validated reverse phase high performance liquid chromatographic technique

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Abstract

Aim: An approach for Simultaneous estimation of rosuvastatin calcium and hydrochlorthiazide from bulk and commercial products using a validated reverse phase high performance liquid chromatographic technique. **Materials and Methods:** The separation of both the drugs was achieved on ACE C_{18} AR (AR - Aromatic) column (250 × 4.6 mm, 5 µm) column using a mobile phase of sodium perchlorate buffer solution (at pH 3.0): Acetonitrile (60:40 v/v). The flow rate was 1 ml/min and detection was done at 280 nm. **Results:** The retention time for Hydrochlorothiazide was 3.9 min and for Rosuvastatin calcium was 10.3 min. Rosuvastatin calcium and Hydrochlorothiazide showed a linear response in the concentration range of 5-30 µg/ml and 6.25-37.5 µg/ml respectively. The correlation co-efficients for Rosuvastatin calcium and Hydrochlorothiazide were 0.9998 and 0.9999 respectively. The percentage recoveries obtained for Rosuvastatin calcium and Hydrochlorothiazide range from 99.3% to 100.4% and 99.2% to 100.4% respectively. The results of analysis have been validated as per the International conference on Harmonisation (ICH) guidelines. **Conclusion:** Validation results indicated that the method shows satisfactory linearity, accuracy, precision, and ruggedness. The extremely low flow rate, simple mobile phase composition makes this method cost-effective, rapid, and non-tedious and can also be successfully employed for simultaneous estimation of both drugs in commercial products.

Key Words: HPLC, hydrochlorothiazide, rosuvastatin calcium

INTRODUCTION

Rosuvastatin calcium [Figure 1] is chemically, bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt. Rosuvastatin calcium is a statin drug used as HMG coenzyme A reductase inhibitor for controlling hyperlipidemic conditions. The literature survey reveals that Rosuvastatin calcium was analyzed by the spectrophotometric, HPTLC, and Reserve phase High performance liquid chromatography (RP-HPLC) methods.[1-8] hydrochlorothiazide [Figure 2] is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide. It is used as a first-line calcium-sparing thiazide diuretic drug that acts by inhibiting the kidneys' ability to retain water. This reduces the cardiac output and hence causes

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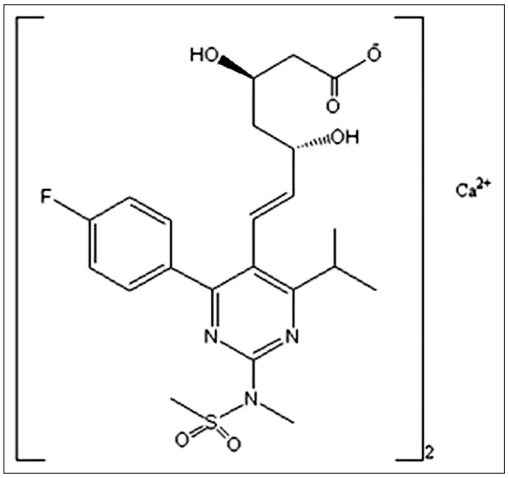

Figure 1: Structure of rosuvastatin calcium

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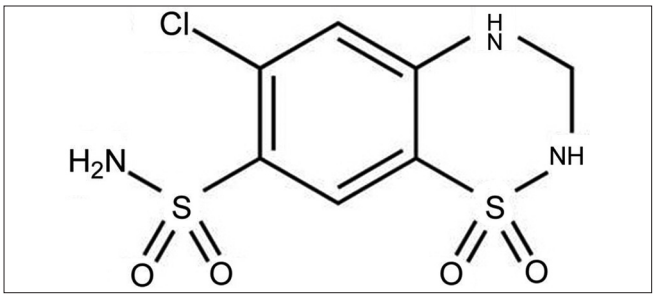

Figure 2: Structure of hydrochlorothiazide

antihypertensive action. Literature survey reveals bio-analytical methods by Liquid chromatography mass spectrometry (LCMS) for detection of hydrochlorothiazide in human serum and blood, few spectrophotometric methods, and HPLC methods for the quantitative estimation of hydrochlorothiazide in bulk and pharmaceutical formulations.[9-16] Currently, most commonly prescribed medicines for cardiovascular diseases are statins and diuretics. The present drug combination has promising effect to control heart diseases. Literature review suggests no analytical methods were reported for simultaneous analysis in combined form and hence the following work was carried out.

MATERIALS AND METHODS

Active Pharmaceutical Ingredients (API) Reference standards of Rosuvastatin calcium and hydrochlorothiazide were received from Intas pharmaceuticals Ltd., Ahmedabad, Gujarat. Rosuva-H Tablets were obtained from Zydus Cadila healthcare Ltd., Moraiya, Ahmedabad. Dose of Rosuvastatin calcium was 10 mg and hydrochlorothiazide was 12.5 mg in Rosuva-H Tablets. Acetonitrile (HPLC Grade) used was purchased from Merck (India) Ltd., Mumbai. Orthophosphoric acid and sodium perchlorate were purchased from Spectrochem, India. RP-HPLC was performed using shimadzu HPLC system Liquid Chromatography (LC) 2010HT, Shimadzu Corporation, Japan) equipped with quaternary pump, auto injector, column oven, and Photo diode array (PDA) detector.

Chromatographic Condition

Column: ACE C_{18} AR 4.6 × 250 mm, 5 μm.
Detector: 280 nm.
Injection volume: 10 μl.
Flow rate: 1.0 ml/min.
Temperature: 30°C.
Run time: 12 min.
Mobile phase: Sodium perchlorate Buffer (at pH 3.0): Acetonitrile (60:40).
Diluent: Methanol.

Buffer Preparation

Accurately weighed 4.3 g sodium perchlorate was dissolved in to 1000 ml milli-q water and 1 ml triethylamine was added to this buffer solution, then the pH was adjusted to 3.0 with ortho-phosphoric acid.

Preparation of Standard Solution

Approximately 20 mg of Rosuvastatin calcium and 25 mg of hydrochlorothiazide reference standards were accurately weighed and transferred to a 100-ml volumetric flask. The weighed sample was dissolved in diluent and sonicated for 10 min. The weighed sample was made up to volume with diluent to produce a standard stock solution. Aliquots from the standard stock solutions were appropriately diluted to 100 ml with diluent obtain final standard concentration of Rosuvastatin calcium (20 ppm) and hydrochlorothiazide (25 ppm) respectively.

Preparation of Test Solution

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 20 mg of Rosuvastatin calcium and 25 mg of hydrochlorothiazide was transferred to a 100-ml volumetric flask. A few ml of diluent was added to above flask and the flask was sonicated for 30 min. The solution was made up to the mark with same diluent. Appropriate volume of aliquot was transferred to 100-ml volumetric flask and volume was made up to the mark with diluent to obtain the final standard concentration of Rosuvastatin calcium (20 ppm) and hydrochlorothiazide (25 ppm). The test solution was filtered through 0.45-µ PolyVinyliDene Fluoride Millipore Filter (PVDF) and analyzed by using HPLC.

Method Optimization

The detection wavelength of 280 nm was chosen in order to achieve a good sensitivity for the quantitative determination of Rosuvastatin calcium and hydrochlorothiazide in solid dosage form. The retention time for hydrochlorothiazide was 3.9 min and for Rosuvastatin calcium was 10.3 min. The isocratic program throughout HPLC method was adopted to analyze both components in a single run as shown in [Figure 3].

Method Validation

Validation was carried out with respect to various parameters, as required under ICH guideline Q2 (R1) The developed method was validated with respect to parameters such as linearity, precision, accuracy, specificity, ruggedness, robustness, and solution stability.[17]

SYSTEM SUITABILITY AND SYSTEM PRECISION

The results of system suitability and system precision are presented in Table 1.

Linearity

Appropriate aliquots of Rosuvastatin calcium and hydrochlorothiazide standard stock solutions were taken in different 100-ml volumetric flasks. The volume was made up to the mark with diluent to yield solutions in the final concentration range of 5-30 µg/ml for Rosuvastatin calcium and 6.25-37.5 µg/ml for hydrochlorothiazide. The solutions were analyzed using HPLC. Calibration curve for both the drugs are shown in [Figures 4 and 5]. The results of linearity are presented in Table 2.

Precision

The method precision was done by preparing six different sample preparations by one analyst. The results are presented in Table 3. The results obtained were within 2% Relative Standard Deviation (RSD).

Ruggedness

Ruggedness test was determined between different analyst, instrument, and Column. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results are presented in Table 3.

Accuracy

The agreement between the theoretical added sample amount to the placebo and practically achieved sample amount from placebo has been employed for the determination of accuracy of the analytical method. It was achieved at three different levels, i.e., 50%, 100%, and 150% of the target concentration in triplicate. The results are presented in Table 4.

Solution Stability

The standard and sample solutions were found stable up to 24 hr at room temperature.

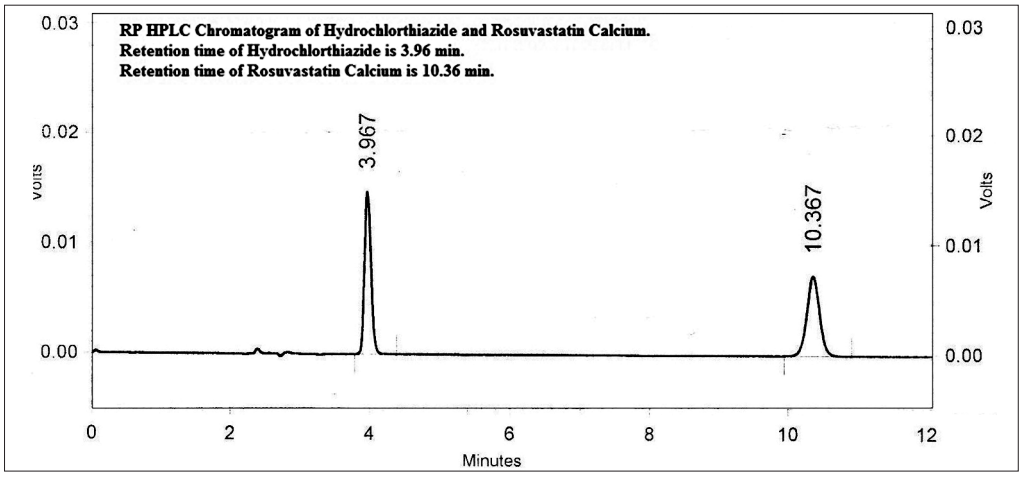

Figure 3: High performance liquid chromatography chromatogram of rosuvastatin calcium and hydrochlorothiazide

Table 1: System suitability results

Drug	Retention time	Theoretical plates	Asymmetry	Resolution
Hydrochlorothiazide	3.9±0.0028	10351	1.09	-
Rosuvastatin calcium	10.3±0.0037	9619	1.12	7.1

Table 2: Linearity data of rosuvastatin calcium and hydrochlorothiazide

Linearity range (%)	Stock solution used for linearity (ml)	Diluted to volume (ml)	Final conc. rosuvastatin (ppm)	Rosuvastatin area	Final conc. Hydrochlorothiazide (ppm)	Hydrochlorothiazide area
25	2.50	100	5	46519	6.25	101236
50	5.00	100	10	98026	12.50	202513
75	7.50	100	15	150543	18.75	303845
100	10.0	100	20	205653	25.00	405236
125	12.5	100	25	260541	31.25	505236
150	15.0	100	30	315623	37.50	612361

Robustness

Robustness of the method was carried out by deliberately made small changes in the flow rate, pH, and organic phase ratio and column oven temperature. Results are presented in Table 5.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD is defined as the lowest concentration of an analyte that can be reliably differentiated from background levels. LOQof an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ of Rosuvastatin calcium and hydrochlorothiazide is calculated based on the standard deviation (SD) of response and slope of calibration curve using formula.

LOD = σ/S × 3.3

LOQ = σ/S × 10

(where σ is Standard deviation of response and S is slope of calibration curve)

The results of LOD and LOQ are mentioned in Table 6.

Table 3: Precision and ruggedness data for rosuvastatin calcium and hydrochlorothiazide

Parameters	Rosuvastatin calcium		Hydrochlorothiazide	
	% assay mean (*n*=6)	% RSD	% assay mean (*n*=6)	% RSD
Method precision	99.7	0.7	100.1	0.8
Ruggedness	99.5	0.8	100.0	0.9

RSD: Relative standard deviation

Table 4: Accuracy data of rosuvastatin calcium and hydrochlorothiazide

Levels (%)	Rosuvastatin calcium		Hydrochlorothiazide	
	% assay mean (*n*=3)	% RSD	% assay mean (*n*=3)	% RSD
50	100.4	0.7	99.2	0.6
100	99.3	0.6	99.5	0.5
150	100.4	0.5	100.4	0.2

RSD: Relative standard deviation

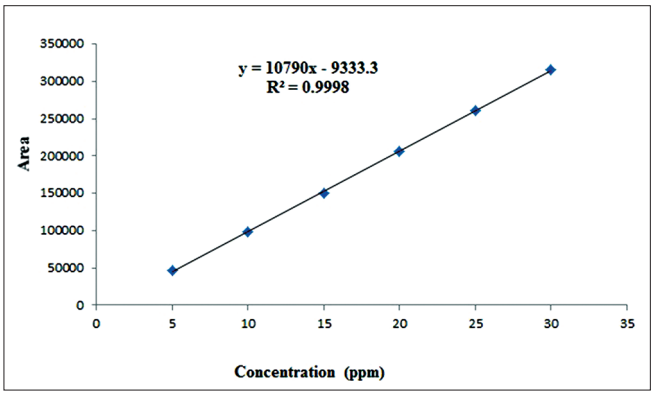

Figure 4: Calibration curve of rosuvastatin calcium

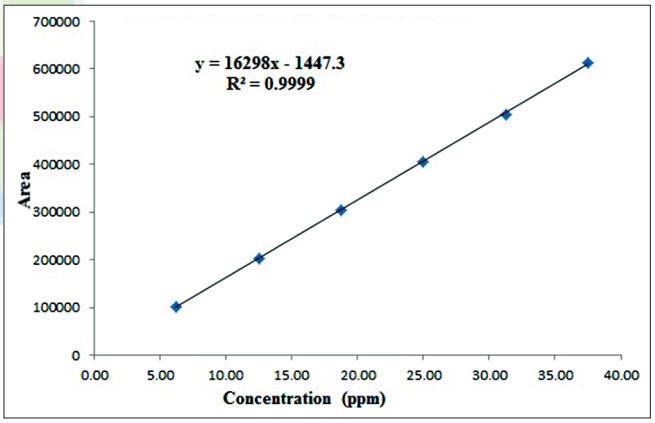

Figure 5: Calibration curve of hydrochlorothiazide

Table 5: Robustness data for rosuvastatin calcium and hydrochlorothiazide

Robustness study			
Changing factor	**Level**	**Rosuvastatin calcium (*n*=6) mean % assay and % RSD**	**Hydrochlorothiazide (*n*=6) mean % assay and % RSD**
Column temperature	25°C	99.9 (0.1)	99.1 (0.9)
	35°C	99.9 (0.1)	99.5 (0.5)
Flow rate	1.1 ml/min	99.9 (0.1)	99.3 (0.7)
	0.9 ml/min	99.2 (0.8)	99.1 (0.9)
Organic mixture ratio to buffer (change in organic mixture composition of mobile phase upto 2%)	Buffer:Acetonitrile (62:38)	99.8 (0.2)	99.7 (0.3)
	Buffer:Acetonitrile (58:42)	99.3 (0.7)	99.1 (0.1)
pH of buffer used in mobile phase	pH 3.2 buffer	99.3 (0.7)	99.7 (0.3)
	pH 2.8 buffer	99.4 (0.6)	99.8 (0.2)

RSD: Relative standard deviation

Table 6: Summary of validation parameters

Parameters of validation	Acceptance criteria	Rosuvastatin calcium	Hydrochlorothiazide
Linearity	Covers the entire range	5-30 µg/ml	6.25-37.5 µg/ml
Correlation coefficient	Correlation coefficient r^2 >0.9999	0.9998	0.9999
LOD	S/N >2 or 3	0.16 µg/ml	0.20 µg/ml
LOQ	S/N >10	0.48 µg/ml	0.60 µg/ml
Precision (%)	RSD <2	0.7	0.8
Ruggedness (%)	RSD <2	0.8	0.9
Accuracy (%)	Recovery 98-102	99.3-100.4	99.2-100.4
Specificity	No interference of blank	Complies	Complies
Solution stability	>12 h	Stable for 24 h % RSD=0.7%	Stable for 24 h % RSD=0.9%
Robustness	RSD NMT 2% in given condition	Complies	Complies

LOD: Limit of detection, LOQ: Limit of quantization, RSD: Relative standard deviation, NMT: Not more than

RESULT AND DISCUSSIONS

The values of relative standard deviation are satisfactorily low and recovery was close to 100%, which indicated accuracy and reproducibility of methods.

CONCLUSION

Thus, the proposed method was found to be simple, accurate, and precise for the routine analysis of Rosuvastatin calcium and hydrochlorothiazide in the Pharmaceutical solid dosage form by simultaneously adopting this validated method.

REFERENCES

1. Sankar GD, Babu JP, Kumar AB, Krishna VM. RPHPLC method for the estimation of rosuvastatin calcium in bulk and pharmaceutical dosage form. Acta Ciencia Indica Chem 2007;33:1-4.
2. Chaudhari BG, Patel NM, Shah PB. Determination of pravastatin, simvastatin and rosuvastatin by HPTLC. Indian J Pharm Sci 2007;69:130-2.
3. Gupta A, Mishra P, Shah K. A simple UV spectrometric determination of rosuvastatin calcium in pure form and pharmaceutical formulations. E J Chem 2009;6:89-92.
4. Sane RT, Kamat SS, Menon SN, Inamdar SR, Mote MR. Determination of rosuvastatin calcium in its bulk drug and pharmaceutical preparations by high- performance thin layer chromatography. J Planar Chromatogr TLC 2005; 18:194-8.
5. Gupta A, Mishra P, Shah K. UV Spectrometric determination of rosuvastatin calcium in pure form and pharmaceutical formulations. Electronic Journal of chemistry 2009;6:89-92.
6. Gajjar AK, Shah VD. Simultaneous estimation of rosuvastatin and ezetimibe by ratio spectra derivative spectrophotometry method in their fixed combined dosage forms. Int J pharm tech Res 2010; 2:404-10.
7. Available from: http://www.drugs.com/mtm/rosuvastatin.html. [Last accessed 2012 Feb 26].
8. Sevda RR, Ravetkar AS, Shirote PJ. UV Spectrophotometric estimation rosuvastatin calcium and fenofibrate in bulk drug and dosage form. Int J Chem Tech 2011;3:629-35.
9. Available from: http://www.drugs.com/mtm/hydrochlorothiazide.html. [Last accessed 2012 Mar 29].
10. Sagirli O, Onal A, Toker SE, Sensoy D. Simultaneous HPLC analysis of olmesartan and hydrochlorothiazide in combined tablets and *in vitro* dissolution studies. Journal of Chromatography 2007; 66:213-28.
11. Shah NJ, Suhagia BN, Shah RR, Patel NM. Development and validation of a HPTLC method for the simultaneous estimation of Irbesartan and hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci 2007; 69:240-3.
12. González L, López JA, Alonso RM, Jiménez RM. Fast screening method for the determination of angiotensin II receptor antagonists in human plasma by high-performance liquid chromatography with fluorimetric detection. J Chromatogr A 2002;949:49-60.
13. Shah NJ, Suhagia BN, Shah RR, Patel NM. HPTLC Method for the simultaneous estimation of valsartan and hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci 2009;71:72-4.
14. Mehta BH, Morge SB. Simultaneous determination of irbesartan and hydrochlorothiazide by HPTLC method. Indian Drugs 2010;47:71-4.
15. El-Gindy A, Ashour A, Abdel-Fattah L, Shabana MM. Spectrophotometric and HPTLC-densitometric determination of lisinopril and hydrochlorothiazide in binary mixtures. J Pharm Biomed Anal 2001;25:923-31.
16. Vijay MD, Rajaram DS, Kadam SS. Stability indicating HPTLC method for determination of irbesartan in pharmaceutical dosage form. Indian J Pharma Edu Res 2007;41:261-9.
17. European Medicines Agency (emea), ICH Topic Q 2 (R1), Validation of Analytical Procedures: Text and Methodology, June 1995; CPMP/ICH/381/95.

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